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In Vitro Antibacterial Activity of Fluorinated Analogs of Chloramphenicol and Thiamphenicol

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We evaluated the in vitro antimicrobial activity of Sch 24893, Sch 25298, and Sch 25393, three novel analogs of chloramphenicol and thiamphenicol. All of the analogs had minimal inhibitory concentrations of ≤10 μg/ml for 18 chloramphenicol-thiamphenicol-resistant strains of *Shigella dysenteriae* and 21 strains of resistant *Salmonella typhi.* The analogs were also more active than were chloramphenicol and thiamphenicol against chloramphenicol-resistant enteric bacteria, including six strains of *Escherichia coli*, seven strains of *Klebsiella pneumoniae*, and two strains of *Enterobacter cloacae.* Fifty-three strains of ampicillin-resistant *Haemophilus influenzae* were uniformly susceptible to chloramphenicol, thiamphenicol, and the three analogs. Sch 25298 was the most active compound tested (minimal inhibitory concentration, 0.5 μg/ml for all strains). Four of seven chloramphenicol-thiamphenicol-resistant *Haemophilus* strains were susceptible to the fluorinated analogs. Of the three *Haemophilus* strains which were resistant to chloramphenicol, thiamphenicol, and the analogs, two contained less than 10% of the chloramphenicol acetyltransferase activity of the strains which were resistant to only chloramphenicol and thiamphenicol. We conclude that fluorinated analogs of chloramphenicol and thiamphenicol have considerable in vitro activity against a broad spectrum of chloramphenicol-thiamphenicol-resistant, gram-negative bacteria.

We evaluated the in vitro antimicrobial activity of three novel analogs of chloramphenicol and thiamphenicol. All of these compounds have a unique substitution at the 3′ carbon positions; the hydroxyl group present in chloramphenicol and thiamphenicol is replaced by fluorine. Since chloramphenicol and thiamphenicol resistance is usually due to plasmid-mediated production of an acetyltransferase which acetylates the 3′-hydroxyl group (10), it was hypothesized that enzymatic inactivation could not occur with such analogs. These drugs, therefore, should be active against the majority of plasmid-mediated, chloramphenicol-resistant isolates. The three analogs examined were Sch 24893 (the 3-fluoro derivative of chloramphenicol), Sch 25298 (the 3-fluoro derivative of thiamphenicol), and Sch 25393 (the 3-fluoro derivative of thiamphenicol in which the dichloroacetyl group is replaced by a difluoroacetyl group at the R′ position) (Fig. 1).

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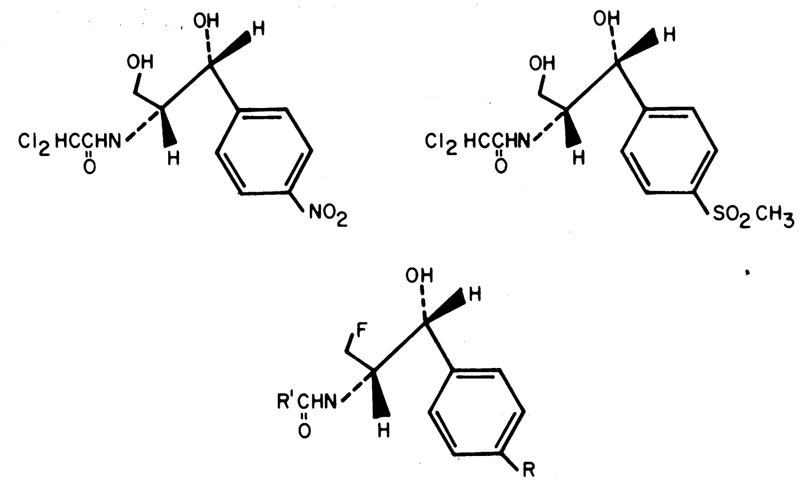
MATERIALS AND METHODS

We used a standard agar dilution technique (2) to determine the minimal inhibitory concentrations (MICs) of chloramphenicol, thiamphenicol, and the three analogs for 53 selected strains of antibiotic-resistant *Enterobacteriaceae* and 60 strains of *Haemophilus influenzae.* Using a Steers replicator (8), 10^4 mid-log-phase organisms were inoculated onto agar containing graded antibiotic concentrations (0.1 to 150 μg/ml). Mueller-Hinton agar was used to test the *Enterobacteriaceae.* The susceptibility of *Haemophilus* was determined with brain heart infusion agar supplemented with β-nicotinamide adenine dinucleotide and defibrinated horse blood as previously described (3). Plates were incubated at 37°C in 85 ± 5% relative humidity without CO_2 supplementation and were examined after 18 h of incubation. Chloramphenicol was obtained in crystalline form from Sigma Chemical Co., St. Louis, Mo. Schering Corp., Bloomfield, N.J., supplied the thiamphenicol and the fluorinated analogs. All compounds except Sch 25293 were dissolved in sterile, glass-distilled water and filter sterilized. Sch 25393 was dissolved in 2 ml of dimethylsulfoxide and diluted to the desired volume with glass-distilled water. When this antibiotic was tested, parallel controls with dimethyl sulfoxide alone were incorporated into the experiment.

Chloramphenicol acetyltransferase activity was as-

CHLORAMPHENICOL THIAMPHENICOL



3-FLUORO ANALOGS

COMPOUND	R	R'
SCH. 24893	NO_2	Cl_2HC
SCH. 25298	SO_2CH_3	Cl_2HC
SCH. 25393	SO_2CH_3	F_2HC

FIG. 1. *Structure of chloramphenicol and thiamphenicol and their fluorinated analogs*

sayed by measuring chloramphenicol-dependent production of coenzyme A from acetyl coenzyme A, using 5,5′-dithiobis-2-nitrobenzoic acid (9, 10). The organisms were grown to mid-log phase (absorbancy of 0.2 at 495 nm) in the absence of chloramphenicol, pelleted at 15,000 × *g*, washed in 1 mM tris(hydroxymethyl)-aminomethane-chloride (pH 7.8) containing 30 mM NaCl, and resuspended in tris(hydroxymethyl)aminomethane-sodium chloride in 1/10 the original culture volume. After sonication in an ice bath (four 30-s pulses at 75 W), the supernatant obtained from a 30-min centrifugation at 30,000 × *g* was assayed for chloramphenicol acetyltransferase activity (10). The protein content of the clarified sonic extract was determined by the method of Lowry et al. (6a), using primary standard-grade bovine serum albumin (Armour, Inc., Chicago, Ill.)

RESULTS

All three analogs were active against the 18 chloramphenicol-thiamphenicol-resistant strains of *Shigella dysenteriae* (Table 1). All of the analogs were susceptible to 1 μg of Sch 25298 per ml. Sch 24893 and Sch 25393 were also active, with MICs of 1 to 5 μg/ml. Twenty-one strains of thiamphenicol- and chloramphenicol-resistant *Salmonella typhi* were also inhibited by the three analogs, although these compounds were slightly less active against *Salmonella* than they were against *Shigella*. Sch 25298 and 25393 were moderately active against all 21 strains, with MICs of 5 μg/ml or less. For Sch 24893, MICs ranged from 5 to 10 μg/ml.

Sixteen other strains of *Enterobacteriaceae* which were highly resistant to both thiamphenicol and chloramphenicol were also tested. Seven of the strains were resistant to 50 μg or more of Sch 24893 per ml, three were similarly resistant to Sch 25298, and two were resistant to Sch 25393. The species of *Enterobacteriaceae* tested are included in Table 1.

We also examined the activity of the three analogs against selected antibiotic-resistant strains of *H. influenzae*. Fifty-three ampicillin-resistant, chloramphenicol- and thiamphenicol-susceptible strains of *H. influenzae* type b were susceptible to the three analogs (Table 2). Sch 25298 was the most active; all strains were inhibited by 0.5 μg/ml. Sch 25393 was the least active; 5 μg/ml was required to inhibit 9 of the 53 strains. Five clinical isolates of *H. influenzae* which were resistant to chloramphenicol, with MICs of >50 μg/ml, were also studied (Table 3). All of these strains were type b except for strain D, which was nontypable. Four of the strains were susceptible to all three analogs, with MICs of 0.1 to 1.0 μg/ml. The three analogs had variable activity against the other three strains, with MICs ranging from 5 to 50 μg/ml. In addition, we studied an *H. influenzae* type b strain (strain

TABLE 1. *MICs of Enterobacteriaceae*

Species	No. of strains	Compound	No. of strains with MIC (μg/ml) of:				
			1	5	10	50	≥100
S. dysenteriae	18	Chloramphenicol					18
		Thiamphenicol					18
		Sch 24893		18			
		Sch 25298	18				
		Sch 25393	1	17			
S. typhi	21	Chloramphenicol					21
		Thiamphenicol					21
		Sch 24893		6	15		
		Sch 25298	1	20			
		Sch 25393		21			
E. coli	6	Chloramphenicol					6
		Thiamphenicol					6
		Sch 24893			5	1	
		Sch 25298		1	4	1	
		Sch 25393			6		
K. pneumoniae	7	Chloramphenicol					7
		Thiamphenicol					7
		Sch 24893		2	1	3	1
		Sch 25298		3	3	1	
		Sch 25393		4	2		1

TABLE 2. *MICs of ampicillin-resistant H. influenzae*

Compound	No. of strains with MIC (μg/ml) of:		
	0.5	1	5
Chloramphenicol	51	2	
Thiamphenicol	20	33	
Sch 24893	32	21	
Sch 25298	53		
Sch 25393	1	43	9

G, Table 3) and one nontypable strain (strain F) with borderline susceptibility to chloramphenicol (MIC, 5 μg/ml at an inoculum of 10^4 colony-forming units). The nontypable strain was susceptible to all three analogs, with MICs of 0.5 μg/ml; the type b strain had MICs of 5 to 10 μg/ml.

The two strains, B and E, which were most resistant to the fluorinated analogs, had negligible chloramphenicol acetyltransferase activity (Table 3). Sonic extracts of these strains contained less than 10% of the chloramphenicol acetyltransferase activity present in the chloramphenicol-resistant but fluorinated analog-susceptibile strains.

DISCUSSION

Studies on the relationship of structure to the activity of chloramphenicol analogs have demonstrated increased activity when the *para* substituent was hydrophobic or if the 2′ side chain was electrophilic (6). Thiamphenicol, the *para*-methylsulfonyl analog of chloramphenicol, is an example of the former; compounds with substitutions on the 2′ carbon are not commercially available. In all studies to date, the 3′-hydroxyl was thought to be essential for biological activity (6). Chemical *O*-methylation or enzymatic *O*-acetylation resulted in compounds without biological activity (5, 6). The essential nature of the 3′-hydroxyl group was reinforced by the finding that R-plasmid-mediated microbial resistance to chloramphenicol was often through the production of 3′-chloramphenicol acetyltransferase (7). Moreover, involvement of the 3′-hydroxyl in an ether linkage, as in the glucuronic acid conjugate, or in an ester linkage, as in the pro-drug succinyl-3′-chloramphenicol, results in the loss of biological activity (4, 5). The 3′-hydroxyl group is thought to stabilise the three-dimensional conformation of the molecule, facilitating its binding to the 50S ribosomal subunit. Fluorine substitution of the 3′-hydroxyl was predicted to preserve the three-dimensional spatial orientation and retain biological activity.

All 53 ampicillin-resistant but chloramphenicol-thiamphenicol-susceptible *H. influenzae* were inhibited by all analogs at concentrations of 5 μg/ml or less, an activity comparable to that of chloramphenicol and thiamphenicol. With chloramphenicol-resistant *S. dysenteriae*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*, the MIC of the fluorinated analog was always less than that of the parent compound. In general, the fluorinated analog of thiamphenicol, and its

TABLE 3. *MICs for chloramphenicol and analogs and chloramphenicol acetyltransferase activity for selected H. influenzae strains*

Strain	MIC for the following compound (μg/ml)					Chloramphenicol acetyltransferase activity (nmol/min per mg of protein)
	Chloramphenicol	Thiamphenicol	Sch 25393	Sch 24893	Sch 25298	
A	50.0	>100	1.0	1.0	0.5	1.54
B	50.0	50.0	50.0	50.0	10.0	0.01
C	50.0	>100	1.0	0.5	0.1	2.06
D	50.0	>100	1.0	1.0	0.5	1.08
E	50.0	5.0	10.0	50.0	5.0	0.08
F	5.0	50.0	0.5	0.5	0.5	0.76
G	5.0	5.0	10.0	5.0	5.0	ND[a]

[a] ND, Assay not performed.

corresponding difluoro isomer, were more active than the fluorinated derivative of chloramphenicol.

Five isolates of *H. influenzae* resistant to chloramphenicol were studied for their susceptibility to the fluorinated analogs. Two were resistant. To gain insight into the mechanism of resistance to analogs lacking the 3′-hydroxyl group, we assayed the chloramphenicol acetyltransferase activity in cell extracts. Those isolates resistant to chloramphenicol, thiamphenicol, and the fluorinated analogs contained less than 10% of the chloramphenicol acetyltransferase activity of the strains which were resistant to chloramphenicol and thiamphenicol alone. The mechanism of resistance in the strains lacking chloramphenicol acetyltransferase may involve cell permeability or a mutation in ribosome-binding proteins (7).

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